Metabolism of Atmospheric Methane in Soils

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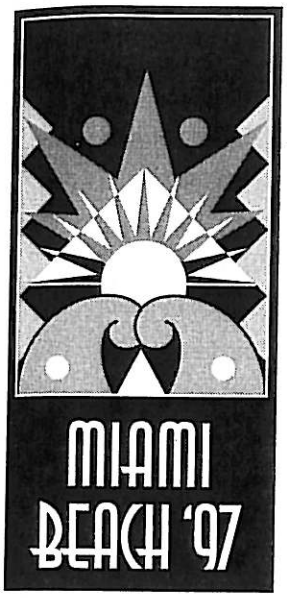

Abstracts
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source utilization was evaluated using a 10-3 dilution on Biolog Gram negative plates; community differences were analyzed using principal components and discriminant analyses. Denitrifying (MPN) bacterial populations were highest in soils with fertilizer and with compost. Nitrifying (MPN) bacteria populations (both ammonium and nitrite) were highest in the soil control and lowest in fertilized soil. There were no differences in culturable soil microbial populations as determined by plate count (TSA and 1/100 TSA) ; total counts were determined by AODC. Microbial community growth characteristics were evaluated by spot plating on cellulose, starch, SAB, and MAC agars. Higher numbers of organisms capable of using cellulose were found in fertilized and control soils; poultry litter-amended soil had the highest fungal populations.

N-104. Propane-oxidizing bacteria from subsoils

Ghaemghami, J., and S. Simkins. Univ. of Massachusetts, Amherst, MA 01003.

Soil bacteria capable of utilizing propane, 1-propanol, and 2-propanol were isolated from subsoils and characterized. Ten Gram-negative, motile bacterial isolates were subjected to Fatty Acid Methyl Ester (FAME) analyses, which found close matches (similarity indices > 70%) for six of the isolates with *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, and Variovorax paradoxus. Three other isolates had similarity indices lower than 35% with Burkholderia pickettii, Comamonas acidovorans, and Chryseobacterium balustinum. The last isolate matched nothing in any database searced and was characterized as a Gram-negative, motile, rod, with a length of 1.2 um. Electron micrographs showed these cells were packed with 0.4-um inclusions that retained their shape and integrity even after the cells were lysed. Further tests such as 16S rRNA sequencing will be undertaken to gain insight into the phylogeny of this organism.

N-105. An Evaluation of Microbial Enzyme Activity as Indicators of Pollution In Stream Sediments.

J. D. CASE,* and P. R. SCHEUERMAN. East Tennessee State Univ., Johnson City

Microbial enzyme activity (MEA) was measured and evaluated for usefulness as a supplement in water quality monitoring in mountainous stream sediments. Sediment was sampled from Upper Higgins Creek in Unicoi County, Tennessee. The Location of Upper Higgins creek is 36B01'00" north and 82B032'30" west. Sediment was sampled monthly at four stations for one year. Three of the stations were selected in areas of the stream that may be subjected to nutrient impact by agricultural activity. The fourth station was located on an adjacent stream and used as a reference. A suite of enzymes including dehydrogenase, protease, amylase, acid phosphatase, alkaline phosphatase, glucosidase, and galactosidase were measured to determine their effectiveness at indicating nutrient impact. Total and organic forms of phosphorus and nitrogen were measured along with the enzymes for comparison. Total plate counts and acridine orange direct counts were performed to monitor the population size and viability in the sediment during the sampling period. Comparisons among the data gathered were made to determine if any significant relationship exists between any of the enzymes and nutrient loading. Correlations between phosphatase activity and organic or inorganic phosphate concentrations were observed. Alkaline phosphatase correlates to organic phosphate with a r=3D0.675, and to inorganic phosphate with a r=3D0.600. Seasonal trends in biomass and enzyme activity were also noted. Glucosidase and galactosidase activity peaked in the fall corresponding to leaf fall. Viable cells and galactosidase correlate with a r=3D0.680. These results indicate that MEAs are useful indicators of changes in water quality in the system studied.

203-N.
Soil and Subsurface Microbiology

Wednesday, 10:45 a.m. — Halls B and C

N-107. Metabolism of Atmospheric Methane in Soils

P. ROSLEV,* N. IVERSEN, and K. HENRIKSEN. Univ. of Aalborg.

The microorganism(s) responsible for consumption of atmospheric methane in soils have never been isolated or characterized. Radiotracer techniques were used to investigate the in situ methane metabolism and to identify potential biomarkers for these unknown methane-oxidizers. The results suggest a limited capacity for microbial growth with atmospheric methane as the sole carbon source. Specific labelling of atmospheric methane-oxidizers with 14CH4 was used to identify signature 14C-phospholipid fatty acids produced by the organisms. The 14C-PLFA fingerprints indicate that consumption of atmospheric methane was due to novel methanotrophic bacteria different from the known methanotrophs. The results also suggest that closely related methanotrophs are likely responsible for atmospheric methane oxidation in diverse soil environments in Denmark, USA and Brazil. The principles of this radiolabelling approach may be applicable to studies of other non-culturable bacteria.

N-108. Role of Methanol in the Oxidation of Atmospheric Methane by Soils and Pure Cultures of Methanotrophic Bacteria

J. B. BENSTEAD (WILLIAMS)* and G. M. KING. Darling Marine Ctr., Walpole, Maine

Methane is oxidized in soils primarily by methanotrophic bacteria; however no known methanotrophs have been shown to grow on atmospheric methane (1.7 ppmv) as the sole carbon and energy source. In the present study, pure cultures of Methylobacter albus BG8 and Methylosinus trichosporium OB3b oxidized atmospheric methane during growth on methanol. This activity was rapidly lost when washed cell suspensions were incubated under atmospheric concentrations of methane in the absence of methanol. In contrast, cultures supplemented with methanol (>5 mM) retained the ability to oxidize atmospheric methane over several days. However, the addition of methanol > 0.2 umol per gfw soil completely inhibited methane consumption within 1h. Inhibition was reversible, since methane uptake resumed after a lag period. Methanol may help support the growth and activity of methanotrophic bacteria in soils by two mechanisms: (I) as a substrate for growth and energy production (II) as a source of reducing equivalents that are required for the continued oxidation of atmospheric methane. However, as high methanol concentrations can be inhibitory, there is a need to assess the physiology of methanotrophic bacteria in the presence of low concentrations of methanol and under conditions of C-limitation that may be more representative of the soil environment.

N-109. Effects of the Methanogenic Inhibitor Lumazine on Methanogenic Substrate Utilization and on Methanotrophic Activity in Alpine Tundra Soils

A. E. WEST* and S. K. SCHMIDT. Univ. of Colorado, Boulder CO.

In soils from the Kobresia plant community of Colorado Rocky Mountain alpine tundra, microbial processes are often water-limited. For example, CH_4 oxidation of these soils is stimulated by water additions in both field and laboratory studies. Previous studies suggested that this stimulation of CH_4 oxidation is a result of transient CH_4 production in anaerobic soil microsites that develop after wetting. We conducted further tests of this hypothesis using lumazine, which is a selective inhibitor of methanogens. Using the SIGR (substrate-induced growth response) method, we tested the effects of lumazine on metabolism of methanogenic substrates in Kobresia soil. Lumazine was added in aqueous solution which was adjusted to pH 7. Salt controls of equal molarity and water controls were also evaluated. Concentrations of 0.5 mM or 1mM lumazine in the soil solution completely inhibited activity of acetate-utilizing microorganisms for 50 or over 100 hours, respectively. Concentrations as low as 0.1 mM significantly delayed growth of acetate-utilizing organisms in soil. Lumazine also reduced activity of methanol-utilizing organisms by approximately 75%. However, lumazine did not have a significant effect on utilization of the non-methanogenic substrate glucose. We also tested the effects of lumazine on soil CH_4 fluxes. At ambient CH_4 concentrations (approximately 1.8 ppm), lumazine additions eliminated observable soil methanotrophy, providing evidence that methanotrophs in this soil may be dependent on endogenous CH_4 production for carbon and energy.

N-110.

Innibition of Denitrification Enzyme Activity by Chloramphenicol.

ROBERT E. MURRAY* and ROGER KNOWLES.

Appalachian State University, Boone, NC and McGill University, Ste.-Anne-de-Bellevue, Canada.

Recent studies have suggested that chloramphenicol used to prevent new protein synthesis during denitrifying enzyme activity (DEA) assays may inhibit existing denitrifying enzymes. We investigated the influence of chloramphenicol on the denitrifying activity of a humisol and a sandy loam soil. Chloramphenicol at a concentration of 0.1g/liter (0.3 mM) decreased DEA between 12.8 and 49.8% although only 3 out of 8 trials were significantly different at the 0.05 level. Chloramphenicol at a concentration of 1g/liter (3 mM) decreased DEA between 28.6 and 55% with 2 out of 3 trials significantly different at the 0.05 level. Chloramphenicol can inhibit existing denitrifying enzymes in mixed populations of soil denitrifiers, although spatial heterogeneity in the distribution of denitrifying activity may mask the effect at low chloramphenicol concentrations.

N-111. Influence of Air Porosity on Distribution of Gases in Soil Cores under Assay for Denitrification

G. W. MCCARTY,* D. R. SHELTON, and A. SADEGHI. USDA-ARS, Beltsville, Md.

There has been concern that short-term measurement of gas emissions from a soil surface may not accurately reflect gas production within the soil profile. But there have been no direct assessments of the errors associated with use of surface emissions for estimating gas production within soil profiles under different water content. To determine the influence of air porosity on the distribution of gases within soil profiles, denitrification assays were performed using soil columns incubated with differ-